Kudoa septempunctata (Myxozoa: Multivalvulida) from the trunk muscle of cultured olive flounder (*Paralichthys olivaceus*) causing food poisoning of human

Hiroshi Yokoyama[1*], Daniel Grabner[2], Sho Shirakashi[3], Ryuhei Kinami[4] and Takahiro Ohnishi[5]

1*:The University of Tokyo, Yayoi 1-1-1, Bunkyo, Tokyo 113-8656, Japan. E-mail: ayokoh@mail.ecc.u-tokyo.ac.jp
2: University of Essen, Universitaetsstrasse 5, D-45141 Essen, Germany.
3: Fisheries Laboratory, Kinki University, Shirahama, Wakayama 649-2211, Japan.
4: Fishery Development Division, Shizuoka Prefecture, Aoi, Shizuoka 420-8601, Japan.
5: National Institute of Health Sciences, Kamiyoga 1-18-1, Setagaya, Tokyo158-8501, Japan.

INTRODUCTION

Recently, cases of a new food-borne disease of human associated with ingestion of olive flounder *Paralichthys olivaceus* have increased in Japan. The patients showed diarrhea and vomiting within 2-20 hours after consumption of raw fish (sashimi), however the prognosis was usually good. Epidemiological analysis and toxicity tests with experimental animals indicated that the causative agent was *Kudoa septempunctata* infecting the trunk muscle of olive flounder (Kawai et al., submitted). In the present study, prevalence and intensity of infection with *K. septempunctata* of cultured olive flounder were investigated by microscopic observation and PCR assay. We aimed to develop a practical control strategy for preventing the food poisoning of consumers.

MATERIALS & METHODS

To determine the prevalence and intensity of infection with *K. septempunctata*, ten fish (ca. 30-35 cm in length) were randomly sampled each time from an infected stock of cultured olive flounder in February, April and May 2011. Pieces of tissue were collected from the opercular muscle using a cotton swab, and presence or absence of spores was quickly checked by wet mount observation (St-Hilaire et al., 1997). Tissues (0.5 g) of the skeletal muscle were collected and passed through mesh screens. After spore suspensions were centrifuged, pellets were re-suspended in PBS, and number of spores were counted in a hemacytometer. DNA was extracted from the skeletal muscle tissue, and PCR assays based on the 28S rDNA were conducted according to the protocols of Grabner et al. (in preparation).

RESULTS

Gross observation

Infection with *K. septempunctata* in fish was subclinical, showing neither cyst formation nor myoliquefaction (Fig. 1).

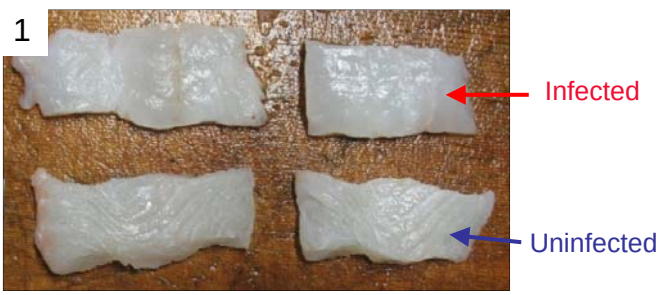
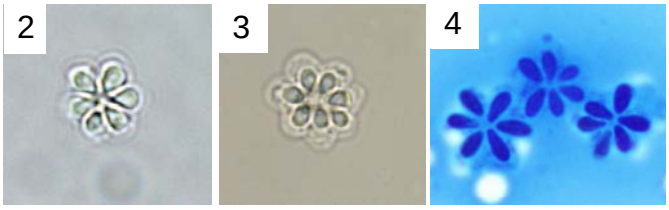


Fig. 1. Slices of fillet from infected (upper ones) and uninfected (lower ones) fish. Infected fillets are visually undistinguishable from uninfected ones.

Light microscopy

Spores of *K. septempunctata* with 5-7 polar capsules (Figs. 2-4) were clearly detected from the opercular and skeletal muscle at a same rate (Table 1).



Figs. 2-4. Fresh spores of *K. septempunctata* with six (**2**) and seven (**3**) polar capsules. Methylene blue-stained spores (**4**).

Prevalence and intensity

Prevalence of infection was 40-50% by light microscopy but 100% by PCR. Intensity of infection (number of spores/g-tissue) varied among individual fish, between 10^4-10^6 spores/g (Table 1).

Table 1. Prevalence and intensity of infection with *K. septempunctata* in the infected lot of olive flounder. nd: not determined.

Month	Prevalence of infection (%)			Intensity of infection in skeletal muscle (no. spores / g-tissue)
	Microscopy		PCR	
	Opercular muscle	Skeletal muscle	Skeletal muscle	
Feb	50	50	100	$2.0 \times 10^4 – 8.5 \times 10^6$
Apr	50	50	100	$1.3 \times 10^4 – 9.7 \times 10^6$
May	40	40	nd	$3.0 \times 10^4 – 5.7 \times 10^6$

DISCUSSION

Subclinical infection with *K. septempunctata* suggests that sashimi containing spores would be eaten without being noticed by consumers. Intensity of infection reached a maximum of 10^6 spores/g, which is high enough to cause food poisoning in human, though lightly infected fish (PCR-positive and microscopy-negative fish) may not be a health problem. Prevention and treatment of myxozoan diseases are generally difficult, and thus it is important to detect and remove infected fish as early as possible from the stock. In the present study, detection of spores from the muscle under the operculum was found to be a simple and practical diagnostic test in fish farms. Early diagnosis of juveniles using the PCR assay and a large-scale screening of commercial-sized fish using the opercular muscle test are recommended to eliminate infected fish prior to their sale on the market. Further studies are required on the quantitative risk analysis (threshold of infection intensity causing food poisoning) to provide a basis for decision-making of selling commercial-sized fish on the market.

REFERENCE

St-Hilaire S, Ribble C, Whitaker DJ, Kent ML (1997) Evaluation of a nondestructive diagnostic test for *Kudoa thyrsites* in farmed Atlantic salmon (*Salmo salar*). *Aquaculture*, **156**, 139-144.